Experimental section

Materials

All reagents used in experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA) with the exception of Amphotericin B, that was purchased from Inlab (São Paulo, Brazil). Poly-L-lysine was of a molecular weight range of 70,000-150,000 in a solution at 0.01%.

Bacterial culture

Cells of *Mv. blakemorei* strain MV-1 were anaerobically cultured in an optimized medium[1] in vials for 48 hours at 28°C before being used in fermentation experiments.

Bioreactor culture

Volumes corresponding to a final cell concentration of 10^8 cells.mL^{-1} were inoculated into a 5-L benchtop bioreactor (2 L working volume) (Minifors, Infors HT-Basel, Switzerland) containing fresh optimized medium. The culture parameters were set as it follows: pH 7.0 (adjusted with 1.0 N NaOH or HCl), stir rate of 100 RPM, temperature 28ºC and undetectable oxygen. The anaerobic condition was achieved by purging sterile nitrogen and in fresh medium until the oxygen sensor reading reached zero. The medium was then purged with N_2O for 15 minutes.

Isolation of magnetosomes

At the end of the growth period in bioreactor, cells were collected by centrifugation at 6.100 × g at 4°C for 15 min. The cell pellets were washed and resuspended in 15 mL of HEPES buffer (10 mM). Afterwards, the cells were lysed in ultrasonic cell crusher (VCX 500, Sonics, Newtown, CT, USA) at 40% amplitude, 20 kHz frequency, in 60 cycles of 30 s between intervals of 30 s. The magnetosomes were magnetically concentrated by a neodymium-boron magnet attached to the outside of the tube for 12 h at 4 °C. The crystals were transferred into 1.5 mL polypropylene tubes and resuspended in HEPES buffer (10 mM) with NaCl (200 mM). The crystals were then washed in an ultrasonic bath (Branson 2200, Emerson, Rochester, NY, USA) for 4 cycles of 30 min, with magnetic concentration and exchange of the buffer at each cycle. The washing efficiency and conservation of the magnetosome membrane were verified by transmission electron microscopy.

Transmission electronic microscopy

Suspensions of pure and functionalized magnetosomes were added on Formvar-coated copper grids and vacuum-dried. Samples were observed in a transmission electron microscope (FEI Morgagni, Hillsboro, OR, USA) operating at 80 kV in magnifications of 16,000 and 42,000 times.

Size measurements of magnetosomes

Measurements of length and width of the magnetosomes used in this study as well as evaluation of the membrane thickness surrounding the magnetosomes before and after functionalization were performed using the iTEM (Olympus, Tokyo, Japan) program. The length and width of the crystals were obtained from the measurements of maximum diameter and minimum diameter, respectively. Graphs and statistical analyzes of the data were carried out with the aid of the Prism 5.0 program (GraphPad Software, San Diego, CA, USA).

Preparation of functionalized nanoparticles

The functionalization of the isolated magnetosomes with amphotericin B were performed by an adapted method[2]. Briefly, 100 μg of magnetosomes were added to 100 μl of 0.1 M phosphate buffer (pH 7.4). Glutaraldehyde was added for crosslinking at different final concentrations (0.2, 3.5 and 12.5% v/v). Amphotericin B dispersed in DMSO was then added to a final concentration of 125 $\mu g.mL^{-1}$. The system was subjected to 5 cycles of 10 minutes sonication at 60 W in a sonicator bath, at 10-min intervals under ice bath cooling. The same procedure was performed with magnetosomes pre-treated with poly-L-lysine at different concentrations (0.1, 0.01 and 0.001 ‰). At the end, the functionalized magnetosomes were magnetically concentrated and the supernatant was removed and used to estimate the capture efficiency of amphotericin B by absorbance at 410 nm. The loading of drug was also calculated from the amount of drug captured and the mass of magnetite added to the functionalization reaction. Aliquots of them were also submitted to transmission electron microscopy observation, as previously described. The experiments were performed in triplicate and the capture efficiency displayed by each system was compared statistically by the ANOVA test using the Prism 5.0 program. The functionalized nanoparticles were vacuum dried and stored frozen at -20°C until used for experiments.

Zeta potential

The zeta potential of resuspended nanoparticles in ultrapure water (30 $\mu g.mL^{-1}$) was measured on a Zeta analyzer (ZetaPlus, Brookhaven Instruments Corp., Holtsville, USA). Ten measurements were performed on each sample and the individual values were used to calculate the mean and standard deviation.

Fourier transform infrared spectroscopy

Lyophilized samples of approximately 1 mg were placed in direct contact with the infrared attenuated total reflection (ATR) diamond crystal of an IRPrestige-21 Spectrometer (Shimadzu, Kyoto, Japan). All preparations were analysed in the wavenumber range of 3000 to 500 cm^{-1} by co-adding 80 scans with a resolution of 1 cm^{-1}.

Magnetic hyperthermia

The heating capacity of the magnetosomes in response to the application of an alternating magnetic field (AMF) was investigated. The analysis was performed on a magnetic induction heating system (DM2-s53, Nanoscale Biomagnetics, Zaragoza, Spain) equipped with a fiber optic temperature probe and vacuum thermal insulation. Suspensions of magnetosomes in PBS (pH 7.4) were transferred to a glass vial (1 mL) at concentrations of 1.2 and 4.8 $mg.mL^{-1}$. The system temperature was stabilized at 22°C for 8 min and the AMF was applied at a frequency of 307 kHz and magnetic field strength of 200 Oe.

Drug release profile

Three types of conjugates were tested for the released profile of amphotericin B. Basically, magnetosome-poly-L-lysine-amphotericin and magnetosome-poly-L-lysine-glutaraledehyde-amphotericin B complexes were dispersed in PBS and incubated at 37°C under agitation at 60 RPM[3]. At 5, 10, 20 and 60 minutes, magnetic nanoparticles were magnetically concentrated and a supernatant sample was collected for the determination of amphotericin B in a spectrophotometer (UV 330G, Gehaka, São Paulo, Brazil) at 410 nm. Thereafter, the magnetosomes were redistributed and the PBS volume was restored. To assess the release of amphotericin B from the nanoparticles in response to AMF, the same procedure was performed for supernatant collection and released drug quantification.

Magnetic measurements

The magnetization properties of magnetosomes was investigated at room temperature using a SQUID vibration sample magnetometer (MPMS3, Quantum Design, San Diego, CA, USA). An amount of 13.9 mg of lyophilized magnetosomes were placed inside a gelatin capsule prior to insertion into SQUID sample holder. Measurement were performed at 300 K.

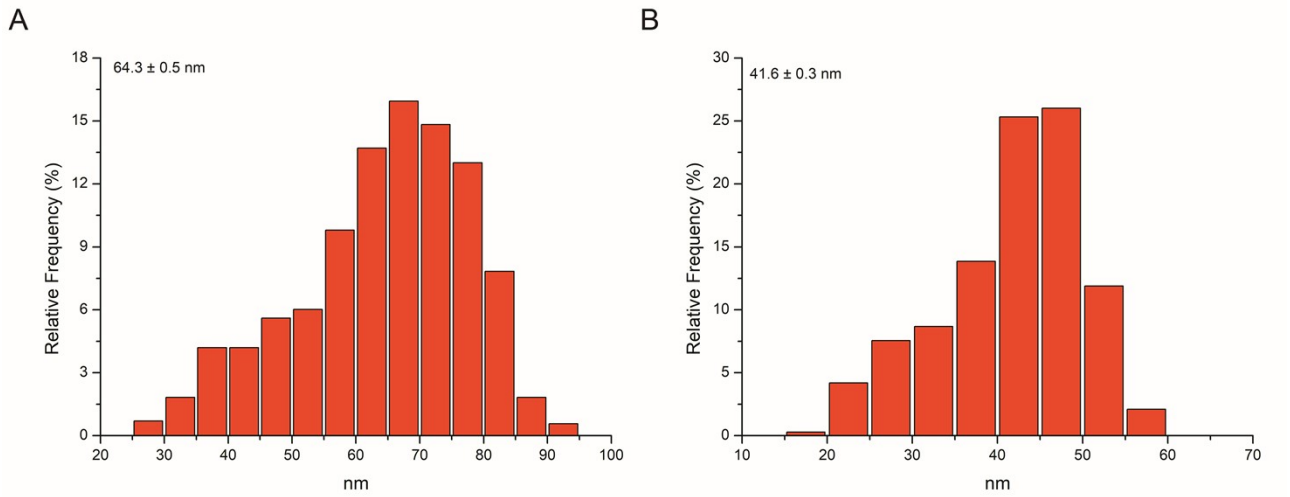
References

1 K. T. Silva, P. E. Leão, F. Abreu, J. A. López, M. L. Gutarra, M. Farina, D. A. Bazylinski, D. M. G. Freire and U. Lins, *Appl. Environ. Microbiol.*, 2013, **79**, 2823–2827.

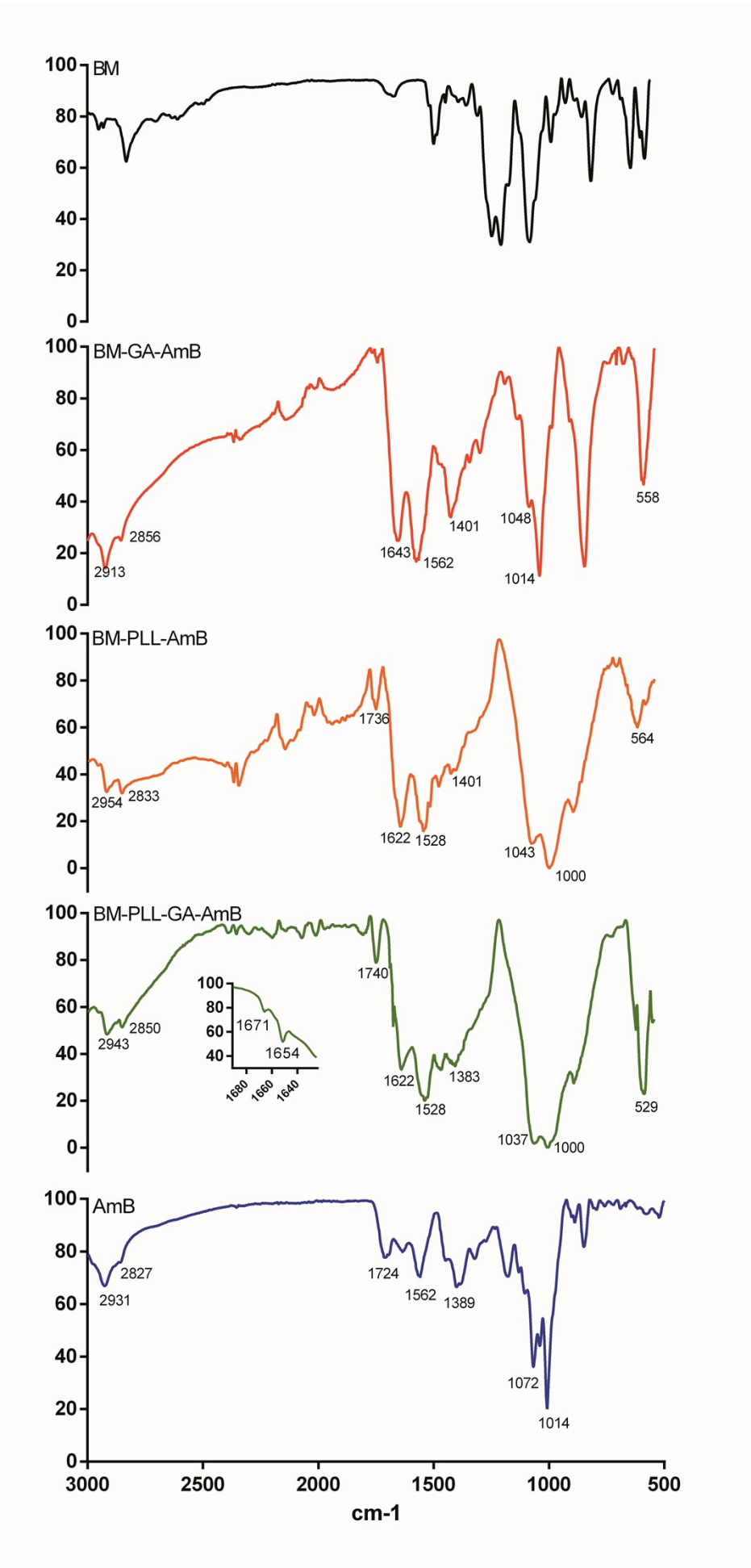
2 J. B. Sun, J. H. Duan, S. L. Dai, J. Ren, L. Guo, W. Jiang and Y. Li, *Biotechnol. Bioeng.*, 2008, **101**, 1313–1320.

3 P. Legrand, M. Chéron, L. Leroy and J. Bolard, *J. Drug Target.*, 1997, **4**, 311–319.

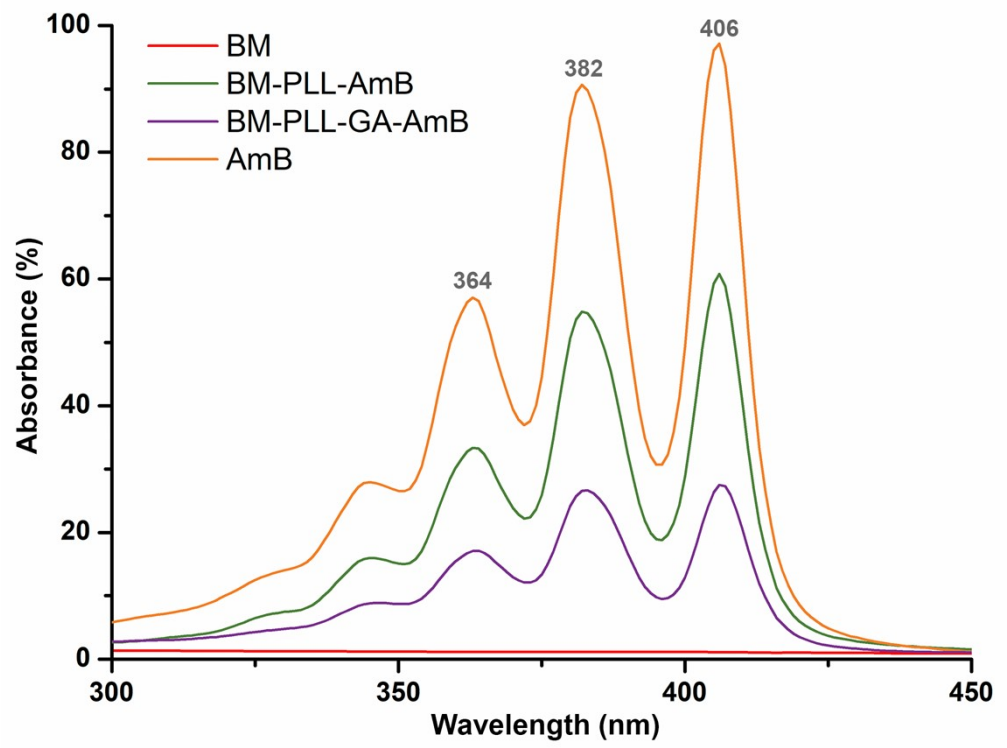
Supplementary figures



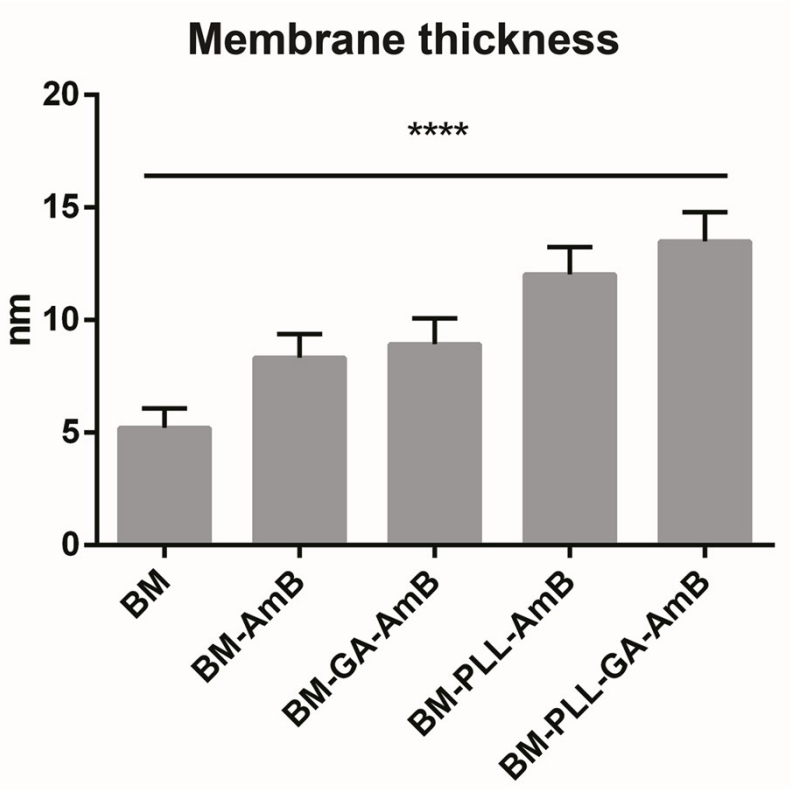
Supplementary figure 1. Length (A) and width (B) distribution histogram (nm) for prismatic magnetosomes from Mv. blakemorei strain MV-1. *All magnetosomes used here are from a single fermentation batch.*



Supplementary figure 2. Fourier transform infrared absorption spectra of BM and conjugates showing peaks indicating functionalization. BM: bacterial magnetite nanoparticles; GA: glutaraldehyde; PLL: poly-L-lysine; AmB: amphotericin B.



Supplementary figure 3. UV-Vis spectra of preparations BM-PLL-AmB and BM-PLL-GA-AmB showing the three characteristic absorption peaks of AmB. Note the absence of those peaks in bulk BM sample.



Supplementary figure 4. Average thickness of the magnetosome membrane for purified samples (control) and for each preparation (BM-AmB; BM-GA-AmB; BM-PLL-AmB; BM-PLL-GA-AmB; BM: bacterial magnetite nanoparticles; GA: glutaraldehyde; PLL: poly-L-lysine; AmB: amphotericin B) ($n \geq 60$).